



# Miniaturized, green salting-out liquid–liquid microextraction coupled with GC–MS used to evaluate biogenic amines in wine samples

M. Fabjanowicz<sup>a,\*</sup>, A. Różańska<sup>a</sup>, K. Kalinowska<sup>a</sup>, J. Płotka-Wasyłka<sup>b</sup>

<sup>a</sup> Department of Analytical Chemistry, Chemical Faculty, Gdańsk University of Technology (Gdańsk Tech), 11/12 G. Narutowicz St., 80-233 Gdańsk, Poland

<sup>b</sup> Department of Analytical Chemistry, Chemical Faculty and BioTechMed Center, Gdańsk University of Technology (GUT), 11/12 G. Narutowicz St., 80-233 Gdańsk, Poland

## ARTICLE INFO

### Keywords:

Gas chromatography  
Box - Behnken design  
Food composition and food authenticity  
Food processing  
Food safety  
Healthy and sustainable diets

## ABSTRACT

Monitoring of the biogenic amines (BAs) content in food products, including wine, is important due to the health and safety of consumers and from the quality control point of view. In a given study, simultaneous derivatization and salting-out liquid–liquid microextraction (SALLME) coupled with GC–MS for BAs determination from wine samples was developed. For the optimization the Box-Behnken design was applied, where three independent factors were evaluated: the amount of NaCl, amount of EtAc and vortexing time. The proposed approach is characterized by good sensitivity expressed in the detection limit between 1.5 and 8.1 µg/L, good recovery on the level 84 – 106% and good reproducibility, it is fast (20 min) and easy to perform. Moreover, the BAs index (BAI) was applied in order to assess the quality of examined wines. BAI value for most of wine samples were below 2 mg/L what indicates that selected wines were of high quality.

## 1. Introduction

Biogenic amines (BAs) are a group of low molecular weight, organic compounds that can be found in raw, processed and fermented food. They are formed in the process of amino acids decarboxylation as a result of natural metabolic activity in animals and plants cells, but also by microorganisms as their protection against the acidic environment [1,2].

There are two main factors influencing BAs content in wine: raw materials (including grape varieties and degree of their maturation, condition of vines cultivation like soil type, fertilization irrigation, climatic condition) and production process (including microbial species and their growth, ageing and fermentation condition) [1,2].

Different grape species have different content of amino acids and BAs. The chemical composition of must formed from grapes has a significant effect on the wine composition. Around 30% of BAs found in grapes are present in the final wine product. Additionally, soil type, climatic condition, fertilization, irrigation, solar radiation all these factors influence vine growth and fruit ripening. Prolonged time of grape ripening causes the raise of phenolic and aroma compounds, as well as free amino acids accumulation (Guo et al., 2015).

Taking into account the production process, one of the most

influential factors on BAs content in wine is a pH value. Given parameters should be adjusted in such a way as to create favorable conditions for microorganisms to carry on malolactic fermentation and prevent the production of BAs. Thus, the pH should be between 3.2 and 3.7. Below 3.2 the malolactic fermentation is disturbed while above 3.7 intensive growth of bacteria is observed which results in a BAs formation. Another factor that may influence BAs content in wine is the prolonged time of skin presence during the maceration process, which may potentially increase their concentration in wine. What is more, the ageing process can contribute to the increased level of BAs in a given alcoholic beverage due to extracellular decarboxylation on the way of protein hydrolysis to amino acids and BAs [2].

Consumption of trace amounts of BAs plays an essential role in numerous physiological functions of the human body. They are acting as hormones and neurotransmitters being responsible for many body functions such as smooth muscle modulation, mucus and gastric acid secretion. Additionally, some of the ingested BAs can be detoxified by acetylation or oxidation. Although, human organism can get rid of small excesses, excessive consumption can be harmful. They can cause adverse effects like headache or gastric and intestinal problems. What is more, in the presence of nitrates and with the increased temperature secondary BAs like spermine, spermidine, tyramine, putrescine or cadaverine may

\* Corresponding author.

E-mail address: [magfabja@student.pg.edu.pl](mailto:magfabja@student.pg.edu.pl) (M. Fabjanowicz).

<https://doi.org/10.1016/j.microc.2022.107616>

Received 16 May 2022; Accepted 19 May 2022

Available online 23 May 2022

0026-265X/© 2022 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

generate nitrosamines that act as carcinogenic agents [3]. However, it is important to monitor the content of BAs in wine samples not only from the health and safety of consumers but also from the quality control point of view. Hence, the presence of BAs can be used as an indicator of spoilage [1].

The aim of a given work was to develop and validate an extraction technique suitable for BAs determination in wine samples with the use of gas chromatography (GC) coupled with mass spectrometry (MS). The focus was paid to create a robust, fast and miniaturized method that can be applied in the industry in order to monitor BAs content in given alcoholic beverages. Taking into consideration, the importance of environmental protection, the development process of salt assisted liquid–liquid microextraction technique (SALLME) was designed in order to minimize its influence on the environment and thus, to meet the criteria of green chemistry. Methodology based on SALLME–GC–MS allows for simple and fast determination of BAs in wine sample. The evaluation of selected BAs provided the basic knowledge about the determination and monitoring of BAs index (BAI) which is important in order to assess the quality of examined wines.

## 2. Materials and methods

### 2.1. Reagent and materials

All BAs: methylamine (MET), dimethylamine (DIMET), ethylamine (ET), diethylamine (DIET), propylamine (PROP), butylamine (BUT), isopentylamine (IPA), hexylamine (HEX), aniline (IS), 2-phenylethylamine (2-PE), putrescine (PUT), cadaverine (CAD), histamine (HIS), tyramine (TYR), tryptamine (TRYP) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Aniline was used as an internal standard. During the analysis with the GC–MS method following chemicals were used: ethyl chloroformate (ECF), used as derivatization agent, purchased from Sigma-Aldrich (St. Louis, MO, USA), ethyl acetate (EtAc) purchased from MERCK, triethylamine (TEA) purchased from Sigma-Aldrich (St. Louis, MO, USA); NaOH purchased from POCH (Gliwice, Poland); NaCl purchased from POCH (Gliwice, Poland). Stock solutions of BAs (1 mg/ml), as well as internal standard (1 mg/ml), were prepared in ultrapure water and stored in 4 °C. Appropriate dilutions of stock solution were prepared on daily basis with water. Water (18MΩ cm, TOC < 5 ppm) was ultra-purified in-lab using the MiliQ Plus system (Millipore, Bedford, MA, USA).

**Table 1**  
Wine sample characteristics.

Label	Year	Type of wine	Voivodship	% Alcohol	Grape type	Sugar content
1R	2015	Red	Opolskie	11.0	Regent	dry
2R	2017	Red	Małopolskie	12.0	Regent	dry
3R	2014	Red	Lubelskie	12.1	Rondo	dry
4R	2013	Red	Małopolskie	12.5	Regent	dry
5R	2017	Red	Dolnośląskie	13.5	Dornfelder	dry
6R	2017	Red	Małopolskie	11.0	Rondo	dry
7R	2017	Red	Dolnośląskie	13.5	Pinot Noir	dry
8R	2016	Red	Zachodnio-pomorskie	13.0	Rondo/Regent	dry
9R	2015	Red	Opolskie	11.5	Rondo	dry
10R	2016	Red	Podkarpackie	12.5	Mix of 3 grapes	dry
1 W	2016	White	Lubelskie	12.0	Johanniter	dry
2 W	2017	White	Dolnośląskie	12.0	Riesling	semi-dry
3 W	2016	White	Lubuskie	12.0	Pinot Gris, Riesling, Muscat Ottonel, Gewurztraminer	semi-dry
4 W	2017	White	Małopolskie	12.0	Seyval Blanc, Hibernal, Johanniter, Solaris	semi-dry
5 W	2016	White	Lubuskie	13.0	Pinot Gris	semi-dry
6 W	2016	White	Lubelskie	12.5	Solaris	sweet
7 W	2014	White	Małopolskie	12.0	Bianca	dry
8 W	2017	White	Zachodnio-pomorskie	12.5	Solaris	dry
9 W	2017	White	Podkarpackie	12.0	Mix of grapes	semi-sweet
10 W	2015	White	Podkarpackie	11.5	Mix of 8 grapes	dry
1Ro	2014	Rosé	Małopolskie	10.5	Zweiglet	semi-dry
2Ro	2015	Rosé	Dolnośląskie	10.5	Regent	dry
3Ro	2016	Rosé	Podkarpackie	11.5	A mix of 3 grapes	dry

### 2.2. Sample

For a given study a total of 23 wines (10 red wines, 10 white wines and 3 rose wines) coming from a different region of Poland were analyzed. All samples were stored at room temperature (21 °C) and were protected from light. Characteristics of wine samples used for the analysis are gathered in Table 1.

### 2.3. SALLME and derivatization procedure

In a given study, derivatization and extraction of BAs were done in parallel preceded by filtration (all wine samples were filtrated through 0.45 μm PES syringe filters). To perform derivatization, ECF was used. ECF reacts with BAs and forms stable, volatile carbamate forms. Derivatization of BAs can be performed in the alkaline solution of a stable pH between 10 and 12 [4]. Thus, as the wine samples has a pH < 4, the pH was adjusted with the use of 1 mol/L NaOH. Moreover, TEA was used to facilitate the reaction and help to remove hydrogen chloride from the solution. The addition of TEA significantly reduced the time of reaction and enabled performing the sample preparation at room temperature [5]. Derivatized BAs were extracted from the sample through salting-out liquid–liquid microextraction, in which EtAc was used as a “greener” alternative to the commonly applied extraction solvents. The scheme of the detailed derivatization and extraction procedure is presented in Fig. 1.

### 2.4. Optimization of the extraction process

The extraction process of BAs from wine samples was optimized with the use of the Box-Behnken design (BBD). The application of the given model helped to perform an efficient optimization process. Based on the preliminary research, there were selected three parameters to be optimized with the use of the BBD, these are amount of salt (NaCl was used with a concentration in the range between 5 and 15% w/v); the amount of extraction solvent (EtAc was added in the range of 50 – 250 μL); vortex time (10–90 sec.). The amount of sample (65 μL) and amount of ECF (1.2 μL), TEA (1.2 μL) were constant [6]. The optimization process was performed on the spiked, red wine samples. What is more, the analysis was done in randomized order to prevent the formation of standard error. The coded and uncoded BBD is presented in Table 2.

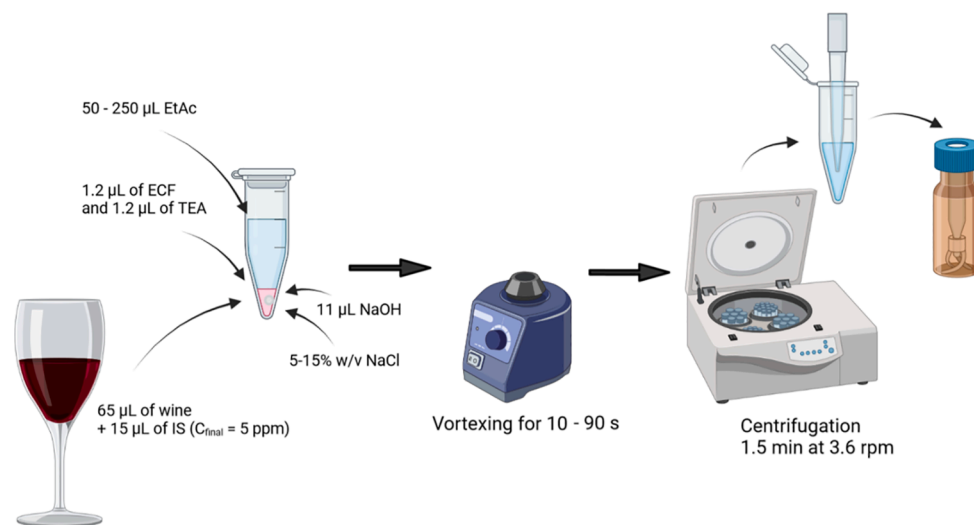


Fig. 1. Schematic representation of the salt assisted liquid-liquid microextraction of BAs from wine samples.

Table 2

Coded and uncoded BBD.

Std Order	Run Order	Coded BBD			Uncoded BBD		
		A – NaCl	B – EtAc	C – Vortex	NaCl [%]	EtAc [µL]	Vortex [s]
1	1	-1	-1	0	5	50	50
2	11	1	-1	0	15	50	50
3	14	-1	1	0	5	250	50
4	12	1	1	0	15	250	50
5	7	-1	0	-1	5	150	10
6	18	1	0	-1	15	150	10
7	2	-1	0	1	5	150	90
8	13	1	0	1	15	150	90
9	17	0	-1	-1	10	50	10
10	9	0	1	-1	10	250	10
11	16	0	-1	1	10	50	90
12	10	0	1	1	10	250	90
13	15	0	0	0	10	150	50
14	3	0	0	0	10	150	50
15	4	0	0	0	10	150	50
16	6	0	0	0	10	150	50
17	5	0	0	0	10	150	50
18	8	0	0	0	10	150	50

## 2.5. GC-MS conditions

The separation of BAs with the use of a GC-MS system was performed on the GC 7890A (Agilent Technologies) system equipped with an electronically controlled split/splitless injection port interfaced to an inert mass selective detector 5975C (Agilent Technologies) with an electron impact ionization chamber. Chromatographic separation was achieved using a ZB-5MS capillary column (30 m × 0.25 mm I.D., 0.25 µm) (Zebtron Phenomenex). The injector temperature (splitless mode) and the interface were set at 250 °C. The sample injection volume was 2 µL. The temperature program was as follows: 55 °C held for 4 min then ramped to 280 °C at 50 °C/min and held for 7.5 min. The total run time was 16 min. Helium was used as the carrier gas at 1.0 mL/min. Spectra were obtained at 70 eV. For better selectivity and sensitivity, the analysis was performed in selected ion monitoring mode (SIM). The ionic fragments of biogenic amines that were detected are presented in Table 3.

## 2.6. Data analysis

MZmine 2 [7] was used to process the chromatographic results. The

Table 3

Characteristic ions for selected biogenic amines.

Amines	Molecular weight [g/mol]	Ions (intensity)
MET	31.05	<b>58</b> (99.9), 75 (47.9), 103 (33.1)
DIMET	45.08	<b>72</b> (99.9), 88 (70.8), 117 (56.0)
ET	45.0	<b>30</b> (99.9), 72 (75.4), 117 (36.3)
DIET	73.14	<b>130</b> (99.9), 145 (44.9), 116 (29.9)
PROP	59.11	<b>102</b> (99.9), 131 (20.4), 103 (6.7)
BUT	73.14	<b>102</b> (99.9), 145 (13.4), 116 (8.4)
IPA	87.17	<b>102</b> (99.9), 90 (33.2), 103 (19.5)
HEX	101.19	<b>102</b> (99.9), 144 (12.2), 90 (11.1)
Aniline (IS)	93.13	<b>165</b> (99.9), 93 (74.0), 106 (61.0)
2-PE	121.18	<b>102</b> (99.9), 104 (46.3), 193 (23.2)
PUT	88.15	<b>102</b> (99.9), 142 (54.3), 141 (18.7)
CAD	102.18	<b>102</b> (99.9), 84 (21.1), 156 (19.4)
HIS	111.15	<b>81</b> (99.9), 154 (51.4), 166 (8.6)
TYR	137.18	<b>120</b> (99.9), 107 (95.2), 102 (31.9)
TRYP	160.22	<b>130</b> (99.9), 143 (38.4), 232 (20.4)

\*quantification ions are marked in bold.

concentrations of BAs in wine samples were calculated and used as input data for multivariate statistical data analysis using the Orange v.3.20 Python toolkit. The first step in the data processing procedure was to standardize the data. The model was then fitted using the Box-Behnken design utilizing a multiple regression analysis (Minitab 17, LLC, State College, Pennsylvania, USA). The model's linear, quadratic, and interaction regression coefficients, as well as their effects, were statistically assessed using analysis of variance (ANOVA) at probability levels ( $p < 0.05$ ).

## 3. Results & discussion

The BBD is one of the most commonly applied experimental design methods used to perform the optimization process. The given model was chosen in order to reduce the number of samples necessary for the optimization process, avoiding experiments under extreme conditions which can lead to unsatisfactory results. The application of BBD helped to save time needed for sample preparation as well as to reduce reagents use and thus the amount of money spent [8]. The number of experiments depends on the number of factors that one can evaluate and is expressed by the following formula:

$$N = 2k(k - 1) + C_0 \quad (1)$$

where N is the number of experiments; k is the number of factors and  $C_0$  is the number of central points [8].

In a given study, three independent factors that influence the extraction of BAs from wine to the organic layer and their interactions were examined, which lead to 12 experiments and 6 central points.

A polynomial model for estimating the BAs in terms of the amount of NaCl added, volume of extraction solvent and time of vortex is shown in the equation (2):

$$\begin{aligned} \text{BAs} = & 1.709 - 0.0178 [\text{NaCl}] - 0.013232 [\text{EtAc}] \\ & - 0.00225 [\text{vortex}] + 0.000038 [\text{NaCl}]^2 + 0.000025 [\text{EtAc}]^2 \\ & + 0.000005 [\text{vortex}]^2 + 0.000084 [\text{NaCl}] \times [\text{EtAc}] \\ & - 0.000023 [\text{NaCl}] \times [\text{vortex}] + 0.000009 [\text{EtAc}] \times [\text{vortex}] \end{aligned} \quad (2)$$

The result of the optimization is presented on the response surface plots in Fig. 2.

The ANOVA test (the results are presented in Table 4) and model coefficient helped to assess the significance of the model. The results of F-value (148.69) and the corresponding p-value indicated that the model was significant.  $R^2$  (0.994),  $\text{adj-}R^2$  (0.987),  $\text{pred-}R^2$  (0.910) high values indicated that the model's form was strongly correlated with the responses and independent variables. Based on the results of p-values of each component, the most influential was the amount of EtAc which obtains p value < 0.001, while the amount of NaCl and vortexing time did not affect the BAs extraction efficiency. The addition of NaCl helped to reduce the solubility of some organic compounds in the aqueous phase, which prevented the formation of emulsion [9]. Even the lowest examined amount of salt (being on the level of 5%) increased the visibility of organic and inorganic layers, no emulsion was observed. Moreover, with the use of the lowest examined amount of EtAc (50  $\mu\text{L}$ ), the best extraction efficiency was obtained. With a given amount of extraction solvent, it was still possible to see two layers and take an appropriate amount of the upper layer for the GC-MS analysis. What is more, the Green Analytical Chemistry principles concerning the decrease of the volume of solvent use and reduction of waste generation can be applied while efficiency is increased. With a given amount of extraction solvent still, it was possible to see two layers and take an appropriate amount of the upper layer for the GC-MS analysis. With the adjusted extraction parameters the figures of merit, presented in Table 5, were evaluated.

According to quality assurance protocol, the following parameters were examined: linearity, precision, sensitivity and accuracy. Linearity was measured with the use of ten aqueous solutions containing selected BAs with concentrations ranging from 0.05 to 1 mg/L and from 1 to 10 mg/L. The given parameter was excellent with the correlation coefficients above 0.99 for all studied compounds. Obtained calibration curves were used for the evaluation of the concentration of the target compounds which was calculated by the ratio of the peak area of the target compound to the peak area of the IS and submitted to the obtained equations from the calibration curves. In terms of precision, both intra- and inter-day precision were determined by the application of five replicates of wine samples spiked at two levels (0.25 and 2.5 mg/L)

**Table 4**

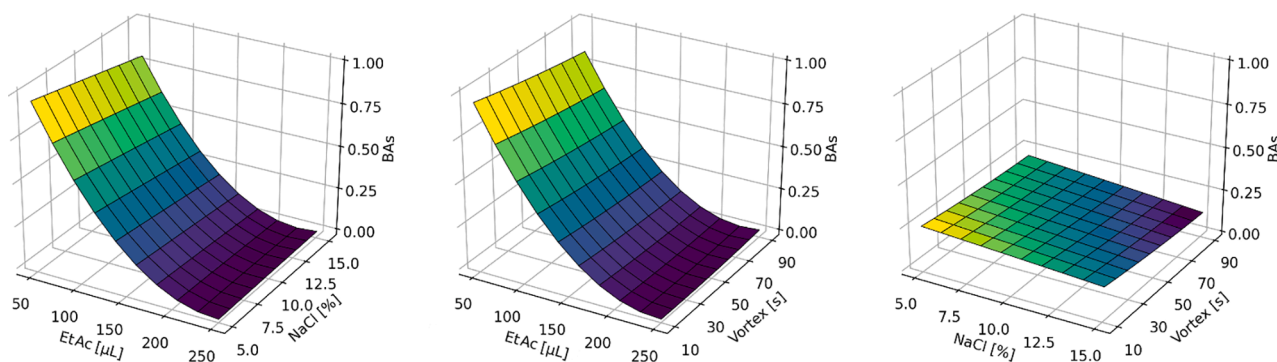
Analysis of variance and regression coefficients of the calculated surface quadratic model for sum of BAs peak areas.

Source	df	SS	MS	F-value	P-value
Model	9	1.81140	0.20127	148.69	<0.001
Linear	3	1.51053	0.50351	371.98	<0.001
NaCl	1	0.00627	0.00627	4.63	0.064
EtAc	1	1.49873	1.49873	1107.22	<0.001
Vortex	1	0.00553	0.00553	4.09	0.078
Square	3	0.28857	0.09619	71.06	<0.001
NaCl*EtAc	1	0	0	0	0.958
EtAc*Vortex	1	0.28113	0.28113	207.69	<0.001
Vortex*Vortex	1	0.00026	0.00026	0.19	0.673
2-Way Interaction	3	0.01231	0.0041	3.03	0.093
NaCl*EtAc	1	0.00706	0.00706	5.22	0.052
NaCl*Vortex	1	0.00009	0.00009	0.06	0.806
EtAc*Vortex	1	0.00516	0.00516	3.81	0.087

\*df – degrees of freedom; SS – sums of squares; MS – mean square.

estimated in the same day for intra-day ( $\text{RSD}_d$ ) precision and on three different days for three weeks for inter-day ( $\text{RSD}_R$ ) precision. Each replicate was submitted to overall methods. For the lower concentration, intra-day precision was between 2.3 and 10 %RSD with the recovery (estimated using the ratio of the peak areas of the spiked samples of known concentration of BAs to those of spiked aqueous solution ( $n = 5$ )) between 85 and 106%, while for higher concentration intra-day precision was between 2.6 and 11 %RSD with the recovery between 89 and 108%. Sensitivity expressed as the LOD and LOQ were calculated from spiked samples ( $n = 5$ ) and the minimum detectable analyte amount with a signal to noise ratio of 3 and 10 were established. LOD was at the level between 1.5 and 8.1  $\mu\text{g/L}$ , the lowest for cadaverine and the highest for putrescine, while LOQ was in the range between 7.6 and 27. Accuracy calculated as a coefficient of variation (CV) was on the level between 82 and 101 %RSD. Exact values of the following validation parameters are gathered in Table 5.

There are several scientific reports, available in the Web of Science and Scopus database, showing different approaches of BAs determination from wine samples, from which 15 are presented in Table 6. From the gathered information it is visible, that the most often applied technique is high-performance liquid chromatography, applied in 8 out of 15 available methods, which involves either a time-consuming extraction process (even up to 55 min) or chromatography separation (even up to 77.5 min). Based on the comparison of the developed method with selected approaches, it can be concluded that proposed method is characterized by satisfactory LOD and recovery values. Moreover, looking at other methods used for BAs determination in wine samples, it can be seen that the given method enables the determination of the highest number of the analytes in a single run (14 BAs) while in the other published scientific reports 4 – 13 analytes, but 13 analytes are determined by GC-MS technique applying longer separation time, 25 min



**Fig. 2.** Response surface plots showing: **A** – the effect of NaCl [%] and ethyl acetate [ $\mu\text{L}$ ] addition; **B** – the effect of the vortex time [s] and ethyl acetate [ $\mu\text{L}$ ] addition; **C** – the effect of the vortex time [s] and NaCl [%] addition on BAs extraction efficiency.

**Table 5**  
Method validation parameters for SALLE-GC-MS.

Analyte	Concentration level				Inter-day (%RSD)			LOD ( $\mu\text{g/L}$ )	LOQ ( $\mu\text{g/L}$ )	ME
	0.25 mg/L		2.5 mg/L		Day 1	Day 2	Day 3			
	Intra-day (% RSD)	Recovery (%)	Intra-day (% RSD)	Recovery (%)						
MET	3.3	91	3.5	95	4.4	4.4	4.6	2.3	7.6	93 (4.1)
DIMET	6.2	93	6.8	97	6.5	6.4	6.7	5.4	18	95 (5.0)
ET	4.1	96	3.3	99	4.8	4.9	4.7	2.3	7.6	96 (9.8)
DIET	10	99	11	100	11	12	12	1.9	6.3	101 (9.3)
PROP	2.3	84	2.6	91	2.5	2.5	2.7	4.2	14	83 (6.9)
BUT	4.7	101	4.9	99	4.3	4.5	4.6	2.8	9.2	95 (7.1)
IPA	3.1	92	3.6	96	3.4	3.1	3.3	5.3	18	96 (8.3)
HEX	6.0	97	5.9	102	6.3	6.6	6.4	2.7	8.9	93 (5.4)
2-PE	4.3	99	5.0	98	4.2	4.4	4.5	6.4	21	94 (7.2)
PUT	4.1	101	4.9	102	4.4	4.7	4.6	8.1	27	98 (3.9)
CAD	2.9	85	3.6	89	3.1	3.0	3.3	1.5	5.0	82 (4.1)
HIS	3.4	95	4.1	97	3.7	3.3	3.7	2.9	9.6	91 (3.7)
TYR	4.7	96	4.9	98	5.0	4.7	4.9	2.1	6.9	94 (9.1)
TRYP	10	106	11	108	11	9.9	11	3.1	10	99 (11)

**Table 6**  
Information on analytical methodologies developed for biogenic amines determination in wine samples.

Separation technique	Sample preparation	Extraction time [min]	Type of derivatizing agent	LODs	Recovery [%]	RSD [%]	Detection	Number of analytes	Time of chromatography analysis [min]	Ref
GC	ion-pair extraction	17	HFB	5 $\mu\text{g/L}$	97,8	<15	MS	7	15	[11]
GC	SPME	50	IBCF	0.009 $\mu\text{g/L}$	85,6	2–11	MS	6	25	[14]
HPLC	filtration	–	DNS-Cl	120 $\mu\text{g/L}$	92,2	2.9–4.2	UV	4	50	[15]
HPLC	UDLLME	46	DMQC-OSu	0.02–5 $\mu\text{g/L}$	95.4–104.6	2.4–5.5	FL	3	10	[16]
HPLC	SPE	55	AQC	15–50 $\mu\text{g/L}$	78.9–120	2.3–6.1	FL	4	47	[17]
MEKC	filtration	–	FBQCA	0.4–12 nM	–	1.5–4.8	LIF	7	15	[13]
RP-HPLC	dSPE	55	Benzoyl chloride	133–509 $\mu\text{g/L}$	72–99	2.5–28.7	UV	12	77,5	[12]
RP-HPLC	LLE	50	CNBF	20–30 $\mu\text{g/L}$	97–102	–	DAD	4	24	[18]
UHPLC	PVPP	45	DNS-Cl	0.02–0.15 mg/L	57.61–109.42	0.72–4.86	UV	8	18	[19]
HPLC	LLE	~37	DNS-Cl	0.25–2.5 $\mu\text{M}$	93.9–106.3	1.6–4.3	FL	7	20	[20]
HPLC	SALLE	27	DNS-Cl	3–28 $\mu\text{g/L}$	–	3.4–25.8	FLD	9	45	[21]
GC	DLLME	10	IBCF/PCF	1.1–3.9 $\mu\text{g/L}$	88–105	2–10	MS	13	25	[10]
GC	SPME	50	IBCF	3.1–25 ng/mL	84–106	2–15	MS	13	25	[22]
CE	–	–	–	15 ng/mL	72–117	2–14	MS	11	30	
GC	SALLME	4	ECF	1.5–8.1 $\mu\text{g/L}$	84–106	2.3–10.4	MS	14	16	This work

GC – gas chromatography; HPLC – high performance liquid chromatography; MEKC – micellar electrokinetic capillary chromatography; SPME – solid phase micro-extraction; UDLLME – ultrasound-assisted dispersive liquid–liquid microextraction; dSPE – dispersive solid phase extraction; LLE – liquid–liquid extraction; PVPP – Polyvinylpyrrolidone; SALLE – salting-out assisted liquid–liquid extraction; DNS-Cl – densyl chloride; DMQC-OSu – 2,6-dimethyl-4-quinolinecarboxylic acid N-hydroxysuccinimide ester; AQC – 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate; CNBF – 2-chloro-1,3-dinitro-5-(trifluoromethyl)benzene (4-chloro-3,5-dinitrobenzotrifluoride); MS – mass spectrometry; UV – UV; FL – fluorescence detection; LIF – laser-induced fluorescence.

(DLLME-GC-MS; Plotka-Wasyłka et al., 2016). Thus, the given method has higher throughput which is sought and preferable in the industry. Additionally, SALLME-GC-MS is the fastest procedure for BAs determination in wine samples. It takes only 4 min for the sample preparation step to be performed and 16 min for the chromatographic separation (20 min in total). Other methods presented in the literature take from 30 min (ion-pair extraction GC-MS; Fernandes & Ferreira, 2000) to even more than 1 h (dSPE-RPLC-UV; Milheiro et al., 2019). There is only one exception where micellar electrokinetic capillary chromatography (MECK) is applied. The analysis lasts only 15 min, however, only seven

analytes are determined in a single run (MECK; ZHANG et al., 2008).

With the application of SALLME-GC-MS, all the compounds of interest were successfully separated and quantified. The information regarding biogenic amines content in wine samples is given in Table 7.

Taking into an account the total BAs content in the group of studied wine samples (red wines: 1173.57  $\mu\text{g/L}$ ; white wines: 612.56  $\mu\text{g/L}$ ; rosé wines 793.54  $\mu\text{g/L}$ ), it is visible that selected red wines are characterized by higher BAs content than white and rosé wines. It relates directly to the production process of the specific type of wine, in the case of red wines it includes a maceration step with grape skin, which results in



Table 7

BAs determined by the SALLME - GC-MS in selected wine samples ( $n = 5$ ).

wines	Analytes [ $\mu\text{g/L}$ ]													
	MET	DIMET	ET	BUT	HEX	2-PE	IPA	TYR	TRYP	PROP	DIET	CAD	PUT	HIS
1 W	<LOD	<LOD	<LOD	<LOD	75.71	31.31	<LOD	<LOD	<LOD	<LOD	<LOD	397	1148	598
2 W	<LOD	$20.41 \pm 0.39$	<LOD	<LOD	$\pm 2.10$ <LOD	$\pm 0.21$ 9.11	<LOD	<LOD	<LOD	<LOD	<LOD	$\pm 10$ 12.09	$\pm 29$ 435	$\pm 11$ 855
3 W	<LOD	<LOD	9.141	<LOD	<LOD	$\pm 0.19$ <LOD	377	<LOD	<LOQ	<LOD	<LOD	$\pm 0.78$ <LOD	$\pm 12$ 317 $\pm$ 11	$\pm 23$ 227.7
4 W	<LOD	$19.32 \pm 0.46$	$\pm 0.039$ <LOD	<LOD	178.2	9.08	$\pm 13$ <LOD	<LOD	<LOD	<LOD	<LOD	32.11	249.1	$\pm 4.6$ 211.1
5 W	<LOD	$29.35 \pm 0.41$	<LOD	<LOD	$\pm 1.9$ <LOD	$\pm 0.19$ <LOD	112.8	<LOD	<LOD	<LOD	<LOD	$\pm 0.16$ <LOD	$\pm 8.1$ 198.2	$\pm 2.1$ 111.3
6 W	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	$\pm 3.7$ <LOD	<LOD	<LOD	<LOD	<LOD	<LOD	$\pm 6.8$ 91.3	$\pm 1.7$ 259.8
7 W	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOQ	<LOD	<LOD	177.1	$\pm 1.1$ 341	$\pm 2.4$ 899
8 W	<LOD	<LOQ	<LOD	<LOD	<LOD	28.34	234.5	<LOD	<LOQ	<LOD	<LOD	$\pm 3.9$ 105.31	$\pm 12$ 509	$\pm 31$ 1443
9 W	<LOD	<LOD	<LOD	<LOD	<LOD	$\pm 0.33$ <LOD	$\pm 9.7$ <LOD	<LOD	<LOQ	<LOD	<LOD	$\pm 0.88$ 154.1	$\pm 16$ 289	$\pm 51$ 877
10 W	<LOD	<LOD	<LOD	<LOD	115.9	19.38	<LOD	<LOD	<LOD	<LOD	<LOD	$\pm 3.8$ 42.19	$\pm 10$ 257.1	$\pm 22$ 311.1
1R	<LOD	<LOD	11.19	<LOD	$\pm 1.1$ <LOD	$\pm 0.21$ 37.44	109.2	119.4	<LOD	<LOQ	20.11	$\pm 0.19$ 70.17	$\pm 6.1$ <LOD	$\pm 3.0$ 763
2R	<LOD	<LOD	$\pm 0.11$ <LOD	<LOD	<LOD	$\pm 0.39$ <LOD	$\pm 1.3$ <LOD	$\pm 1.7$ <LOD	25.13	<LOD	$\pm 0.24$ <LOD	$\pm 0.93$ <LOD	<LOD	$\pm 19$ 126.0
3R	<LOD	<LOD	<LOD	<LOD	<LOD	31.01	214.2	<LOD	$\pm 0.16$ <LOD	54.2	84.2	<LOD	121.1	$\pm 1.3$ <LOD
4R	<LOD	<LOD	<LOD	<LOD	<LOD	$\pm 0.27$ <LOD	$\pm 2.3$ <LOD	27.11	34.0	$\pm 1.3$ <LOD	$\pm 1.0$ <LOD	<LOD	$\pm 1.9$ 58.11	422
5R	112.7	<LOD	<LOD	<LOD	<LOD	30.47	<LOD	$\pm 0.13$ <LOD	$\pm 1.3$ <LOD	<LOD	<LOD	39.33	$\pm 0.67$ 170	$\pm 14$ 137.3
6R	$\pm 1.6$ <LOD	40.12	<LOD	<LOD	<LOD	$\pm 0.31$ <LOD	111.7	10.21 $\pm 0.10$	19.07	<LOQ	18.51	$\pm 0.22$ 55.26	$\pm 1.9$ 696	$\pm 2.1$ 194.1
7R	<LOD	$\pm 0.77$ <LOD	<LOD	<LOD	<LOD	17.19	$\pm 1.5$ <LOD	<LOD	$\pm 0.23$ <LOD	<LOD	$\pm 0.19$ <LOD	$\pm 0.39$ 87.0	$\pm 17$ 154	$\pm 5.4$ 131.2
8R	<LOD	<LOD	<LOD	<LOD	<LOD	$\pm 0.24$ 13.11	<LOD	<LOD	13.71	<LOD	<LOD	$\pm 1.2$ 191.3	$\pm 1.1$ 330	$\pm 2.0$ 122.1
9R	<LOD	<LOD	<LOD	<LOD	<LOD	$\pm 0.11$ <LOD	<LOD	<LOD	$\pm 0.07$ <LOQ	<LOD	<LOD	$\pm 4.2$ <LOD	$\pm 2.8$ 199	$\pm 1.7$ 389.0
10R	52.31	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	33.15	<LOD	<LOD	17.04	$\pm 17$ 258	$\pm 3.7$ 128.0
1Ro	$\pm 0.25$ <LOD	<LOD	<LOQ	<LOD	70.1	<LOD	29.42	<LOD	$\pm 0.19$ <LOD	<LOD	<LOD	$\pm 0.13$ 47.99	$\pm 21$ 832	$\pm 2.4$ 67.17
2Ro	<LOD	<LOD	<LOD	<LOD	$\pm 1.6$ <LOQ	<LOD	$\pm 0.057$ <LOD	<LOD	<LOD	<LOD	<LOD	$\pm 0.17$ <LOD	$\pm 21$ 432	$\pm 0.71$ 298.0
3Ro	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	41.12	$\pm 15$ 389	$\pm 4.7$ 156.7
												$\pm 0.23$	$\pm 12$	$\pm 1.2$

higher polyphenol, amino acids and thus BAs content [23]. Moreover, the production process of red wines consists also of malolactic fermentation which is not often applied during white wine production or is much shorter [23].

Considering different types of wine samples (white, red and rosé wines) there are different trends in individual BAs abundance observed, presented in Fig. 3.

In red wines, five biogenic amines are the most abundant: HIS (111.3 – 1443.0 µg/L), PUT (91.3 – 1148.0 µg/L), CAD (12.1 – 154.1 µg/L), IPA (112.8 – 377.0 µg/L) and HEX (75.7 – 115.9 µg/L). TRYP were present in a concentration range between 3.03 and 6.07 µg/L, DIMET 13.07 – 29.35 µg/L, 2-PE 9.08 – 31.31 µg/L, while ET was quantified only in one red wine sample 3R with a concentration of 9.14 µg/L and TYR, PROP, DIET, MET, and BUT were below the LOD in all the red wine samples.

In white wines, four biogenic amines are present in significantly higher concentrations than others. These are HIS (122.1 – 763.0 µg/L), PUT (58.1 – 696.0 µg/L), CAD (17.04 – 191.30 µg/L) and IPA (109.2 – 214.2 µg/L). The rest of the analyzed biogenic amines were present in much lower concentrations like 2-PE (13.11 – 37.44 µg/L), TYR (10.21 –

119.4), TRYP (9.11 – 33.15 µg/L), PROP (8.36 – 54.2 µg/L) and DIET (18.51 – 84.2 µg/L). MET was quantified only in two wine samples (52.31 – 112.70 µg/L), DIMET and ET were quantified only in one wine sample (40.12 µg/L and 11.19 µg/L respectively) while HEX and BUT were below the detection limit in all white wine samples.

In rosé wines, four biogenic amines are present at the highest concentration, these are PUT (389 – 832 µg/L), HIS (67–298 µg/L), CAD (41–48 µg/L) and HEX (11–70 µg/L). IPA and ET were quantified in only one rose wine sample 1Ro with a concentration of 29.4 and 6.1 respectively, while the rest: TYR, TRYP, PROP, DIET, MET, DIMET, 2-PE were below the detection limit in all studied rose wine samples.

In all the studied Polish wine samples PUT, CAD and HIS were the most abundant BAs regardless of the wine type which is similar to the BAs content reported in wines of different origins. In Greek red wines the most abundant was PUT (0.14 – 5.90 mg/L) followed by CAD (0.12 – 3.94 mg/L), HIS (0.04 – 2.63 mg/L) and TYR (0.10 – 1.75 mg/L). However, in Greek white wines the most abundant was PUT (0.22 – 9.07 mg/L) followed by TYR (0.03 – 1.80 mg/L) and HIS (0.18 – 1.09 mg/L) [24]. Spanish wines were characterized by the BAs profile presented as

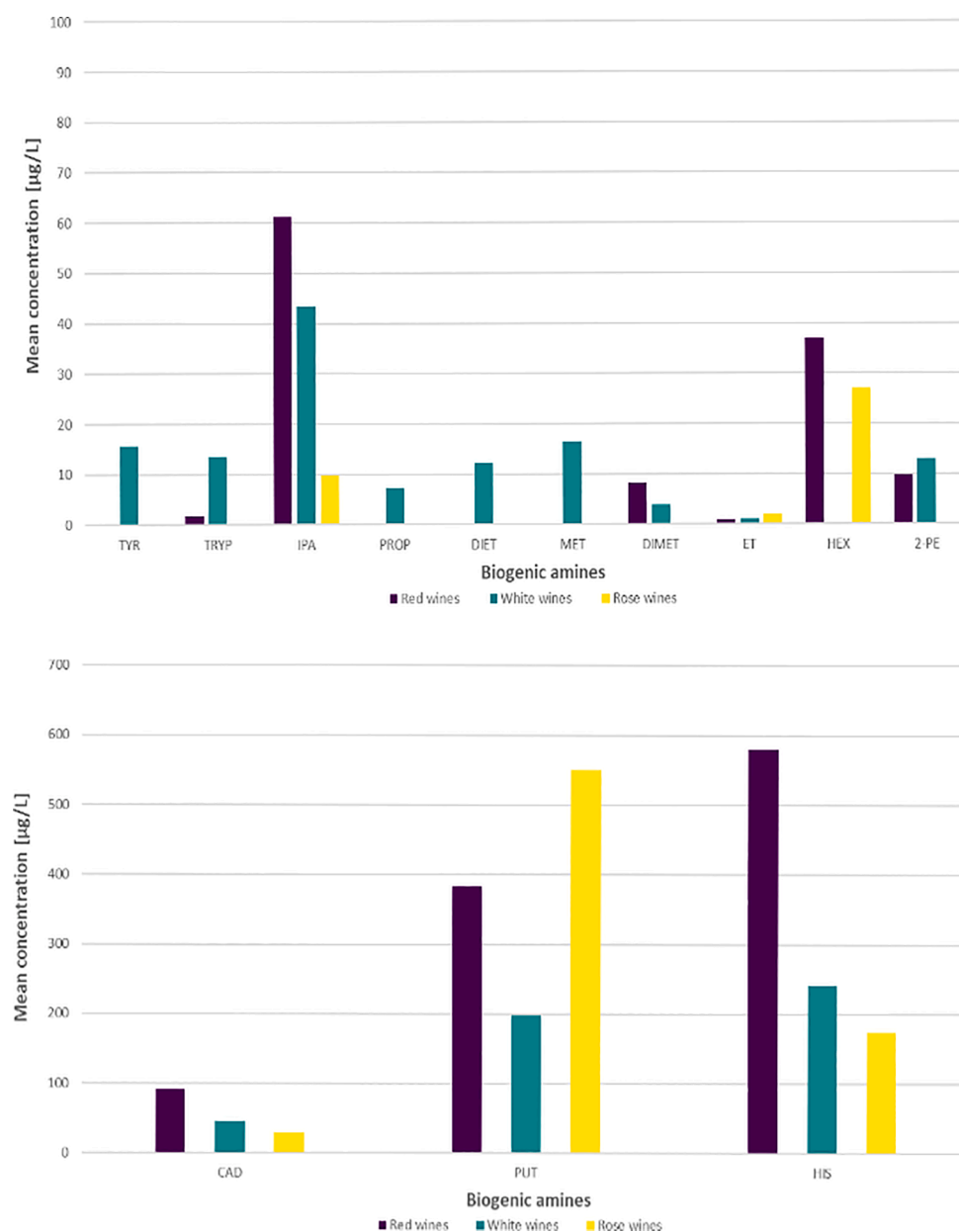


Fig. 3. Mean concentration of the selected BAs in white, red and rosé wine samples.

follows PUT (0.06 – 99.9 mg/L) > TYR (0.03 – 18.2 mg/L) > HIS (0.39 – 15.9 mg/L) > CAD (0.3 – 14.1 mg/L) in red wines and PUT (1.93 – 26.5 mg/L) > TYR (0.53 – 11.3 mg/L) > HIS (0.67 – 10.8 mg/L) in white wines. In Spanish rosé wines, HIS (0.46 – 5.18 mg/L) and PUT (2.64 – 4.01 mg/L) were the most abundant BAs [24]. However, the concentration of the selected BAs was significantly higher than BAs concentration in Polish wines.

The high content of PUT may indicate that the ageing process took place in the oak barrels or that yeast and malolactic bacteria were still present during the ageing process of the selected wines [25]. CAD and TYR are produced by yeast autolysate, which transforms tyrosine and lysine amino acids, added to the must, into corresponding BAs [1]. While histamine, the most often studied BA due to its toxicity, is produced by bacteria and yeast through histidine decarboxylation. Headaches, low blood pressure, heart palpitations, vomiting and diarrhoea are among documented side effects of a given compound. The most vulnerable group of people are patients with histamine intolerance. Taking into account the health and safety of consumers it is very important to monitor the content of a given compound in food and beverages products [1].

### 3.1. Wine quality assessment

There are no established limits for the content of the BAs in wines. There is only one recommendation regarding histamine concentration in wine samples, however, its upper limit varies by country. In Germany, there is 2 mg/L, in Belgium 5–6 mg/L, in France 8 mg/L and Switzerland 10 mg/L allowed content [1]. In all of the examined wine samples, the histamine level was much lower than the most strict acceptance limit determined in Germany. The histamine content in the studied wine samples was on a level between 0.23 and 0.011 mg/L.

The histamine and tyramine are BAs having the most toxic effects on human health, which can be even enhanced in the presence of aliphatic diamines putrescine and cadaverine [26]. Thus, in order to evaluate the quality of food product, there is a BAs index (BAI) introduced [26]. Given index is based on the sum of the concentration (C) of the given four BAs, as follows (Equation (3)):

$$BAI = C_{PUT} + C_{CAD} + C_{HIS} + C_{TYR} \quad (3)$$

The BAI index found its application to assess the quality of meat [27]. The meat of poor hygiene quality has BAI between 20 and 50 mg/kg, while spoiled meat has a BAI value greater than 50 mg/kg [28]. In a given study the BAI index was applied to wine samples in order to assess their quality regarding the chosen four BAs, the results are presented in Fig. 4.

According to the obtained results, the highest BAI value is observed for the red wine samples, which is equal to  $1.1 \pm 0.7$  mg/L. The sum of the concentration of the chosen four BAs in red wine samples varies significantly. However, 75% of obtained results are on the level of 0.35 mg/L and none of the studied red wine samples exceeded 2.4 mg/L. BAI value for white wine samples is equal to  $0.50 \pm 0.28$  mg/L while for rosé wine samples is between 0.56 and 0.95 with a mean value equal to  $0.76 \pm 0.15$  mg/L. This results corresponds to the highest observed total BAs content in the selected red wines. It indicates the usefulness of BAI index in the wine quality assessment. What is more, a similar trend is observed in Italian wine from the region of Abruzia. BAI of Abruzia wine for red wine sample was the highest equal to 16.12 mg/L, while for rosé and white wine samples were much smaller 5.52 and 3.62 respectively [29]. Moreover, the results of BAI values for the wines analyzed in a given study, indicate that studied wine samples were characterized by good quality. They are not spoiled or contaminated by a microorganism, and the production process satisfies the hygienic requirements.

### 4. Conclusions

Carrying out the extraction and derivatization process in parallel

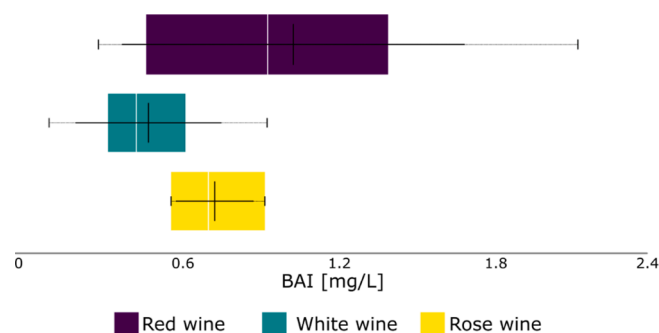


Fig. 4. Box – plot showing BAI values for red, white and rosé group of wines.

prevents the loss and/or contamination of the selected analytes and the reduction of waste generation. What is more, a reduced number of procedure steps leads to time savings. Application of the EtAc as an extraction solvent stands as a green alternative for the commonly applied solvent. The proposed, optimized extraction technique enables efficient BAs extraction with relevant figures of merit that satisfy the requirements of green analytical chemistry. Additionally, comparing given work with other method published in the available databases, it is visible that SALLME-GC–MS stand as a good alternative for BAs determination from wine samples. It enables fast analysis with high throughput with relevant validation parameters what indicates that the given method can be successfully applied for the routine analysis.

### Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

### CRediT authorship contribution statement

**M. Fabjanowicz:** Conceptualization, Methodology, Writing – original draft. **A. Różańska:** Methodology, Writing – review & editing. **K. Kalinowska:** Methodology, Writing – review & editing. **J. Plotka-Wasyłka:** Writing – review & editing, Supervision.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.microc.2022.107616>.

### References

- [1] R.E. Anli, M. Bayram, Biogenic amines in wines, *Food Reviews International*. 25 (1) (2008) 86–102.
- [2] Y.-Y. Guo, Y.-P. Yang, Q. Peng, Y.e. Han, Biogenic amines in wine: a review, *Int. J. Food Sci. Technol.* 50 (7) (2015) 1523–1532.
- [3] R. Tofalo, G. Perpetuini, M. Schirone, G. Suzzi, in: *Encyclopedia of Food and Health*, Elsevier, 2016, pp. 424–429.
- [4] M.A. Munir, K.H. Badri, The importance of derivatizing reagent in chromatography applications for biogenic amine detection in food and beverages, *J. Anal. Methods Chem.* 2020 (2020) 1–14.
- [5] M.J. Paik, Y. Choi, K.R. Kim, Simultaneous profiling analysis of alkylphenols and amines by gas chromatography and gas chromatography-mass spectrometry, *Anal. Chim. Acta* 560 (2006) 218–226, <https://doi.org/10.1016/j.aca.2005.12.047>.
- [6] A. Różańska, M. Fabjanowicz, K. Kalinowska, Ż. Polkowska, J. Plotka-Wasyłka, Green, simple analytical method for biogenic amines determination in fruit juice samples using salting-out assisted liquid-liquid microextraction and gas chromatography-mass spectrometry, *Food Chem.* 384 (2022), 132557, <https://doi.org/10.1016/j.foodchem.2022.132557>.



- [7] T. Pluskal, S. Castillo, A. Villar-Briones, M. Orešič, MZmine 2: Modular framework for processing, visualizing, and analyzing mass spectrometry-based molecular profile data, *BMC Bioinf.* 11 (2010), <https://doi.org/10.1186/1471-2105-11-395>.
- [8] S.L.C. Ferreira, R.E. Bruns, H.S. Ferreira, G.D. Matos, J.M. David, G.C. Brandão, E. G.P. da Silva, L.A. Portugal, P.S. dos Reis, A.S. Souza, W.N.L. dos Santos, Box-Behnken design: An alternative for the optimization of analytical methods, *Anal. Chim. Acta* 597 (2) (2007) 179–186.
- [9] B. Furniss, A. Hannaford, V. Rogers, P. Smith, A. Tatchell, Arthur I, Fourth Edition, Vogel preparatyka organiczna, 1984.
- [10] J. Plotka-Wasyłka, V. Simeonov, J. Namieśnik, An in situ derivatization – dispersive liquid–liquid microextraction combined with gas-chromatography – mass spectrometry for determining biogenic amines in home-made fermented alcoholic drinks, *J. Chromatogr. A* 1453 (2016) 10–18.
- [11] J.O. Fernandes, M.A. Ferreira, Combined ion-pair extraction and gas chromatography–mass spectrometry for the simultaneous determination of diamines, polyamines and aromatic amines in Port wine and grape juice, *J. Chromatogr. A* 886 (1-2) (2000) 183–195.
- [12] J. Milheiro, L.C. Ferreira, L. Filipe-Ribeiro, F. Cosme, F.M. Nunes, A simple dispersive solid phase extraction clean-up/concentration method for selective and sensitive quantification of biogenic amines in wines using benzoyl chloride derivatisation, *Food Chem.* 274 (2019) 110–117.
- [13] N. Zhang, H. Wang, Z. Zhang, Y. Deng, H. Zhang, Sensitive determination of biogenic amines by capillary electrophoresis with a new fluorogenic reagent 3-(4-fluorobenzoyl)-2-quinolinecarboxaldehyde, *Talanta* 76 (4) (2008) 791–797.
- [14] J. Plotka-Wasyłka, V. Simeonov, C. Morrison, J. Namieśnik, Impact of selected parameters of the fermentation process of wine and wine itself on the biogenic amines content: Evaluation by application of chemometric tools, *Microchem. J.* 142 (2018) 187–194.
- [15] Esposito, Montuori, Schettino, Velotto, Stasi, Romano, Cirillo, Level of Biogenic Amines in Red and White Wines, Dietary Exposure, and Histamine-Mediated Symptoms upon Wine Ingestion, *Molecules*. 24 (2019). <https://doi.org/10.3390/molecules24193629>.
- [16] K.-J. Huang, C.-Y. Wei, W.-L. Liu, W.-Z. Xie, J.-F. Zhang, W. Wang, Ultrasound-assisted dispersive liquid–liquid microextraction combined with high-performance liquid chromatography–fluorescence detection for sensitive determination of biogenic amines in rice wine samples, *J. Chromatogr. A* 1216 (38) (2009) 6636–6641.
- [17] A. Peña-Gallego, P. Hernández-Orte, J. Cacho, V. Ferreira, Biogenic amine determination in wines using solid-phase extraction: A comparative study, *J. Chromatogr. A* 1216 (15) (2009) 3398–3401.
- [18] A.M. Piasta, A. Jastrzębska, M.P. Krzemiński, T.M. Muzioł, E. Szyk, New procedure of selected biogenic amines determination in wine samples by HPLC, *Anal. Chim. Acta* 834 (2014) 58–66.
- [19] M.F. Angulo, M. Flores, M. Aranda, K. Henríquez-Aedo, Fast and selective method for biogenic amines determination in wines and beers by ultra high-performance liquid chromatography, *Food Chem.* 309 (2020) 125689.
- [20] N. García-Villar, S. Hernández-Cassou, J. Saurina, Determination of biogenic amines in wines by pre-column derivatization and high-performance liquid chromatography coupled to mass spectrometry, *J. Chromatogr. A* 1216 (36) (2009) 6387–6393.
- [21] R.M. Ramos, I.M. Valente, J.A. Rodrigues, Analysis of biogenic amines in wines by salting-out assisted liquid–liquid extraction and high-performance liquid chromatography with fluorimetric detection, *Talanta* 124 (2014) 146–151.
- [22] M. Woźniakiewicz, A. Woźniakiewicz, P.M. Nowak, E. Kłodzińska, J. Namieśnik, J. Plotka-Wasyłka, CE-MS and GC-MS as “Green” and complementary methods for the analysis of biogenic amines in wine, *Food Anal. Methods* 11 (9) (2018) 2614–2627.
- [23] A. Kántor, M. Kačániová, V. Pachlová, Biogenic amines content in different wine samples, *J. Microbiol. Biotech. Food Sci.* 04 (2015). <https://doi.org/10.1541/4/jmbfs.2015.4.special1.37-40>.
- [24] A. Peña-Gallego, P. Hernández-Orte, J. Cacho, V. Ferreira, High-performance liquid chromatography analysis of amines in must and wine: A review, *Food Rev. Int.* 28 (1) (2012) 71–96.
- [25] F. Harja, M.D. Stănescu, E. Reinhard, K. Korntheurer, I. Popescu-Mitroi, D. Haltrich, C. Peterbauer, Determination of biogenic amines from several romanian wines, accessed May 15, 2022, *Rev. Chim.* 62 (2011) 304–307, <http://bch.ro/pdfRC/HARJA%20F.pdf%203%2011.pdf>.
- [26] M.T. Veciana-Nogués, A. Mariné-Font, M.C. Vidal-Carou, Biogenic amines as hygienic quality indicators of tuna. relationships with microbial counts, ATP-related compounds, volatile amines, and organoleptic changes, *J. Agric. Food. Chem.* 45 (6) (1997) 2036–2041.
- [27] W. Wojnowski, K. Kalinowska, T. Majchrzak, J. Plotka-Wasyłka, J. Namieśnik, Prediction of the biogenic amines index of poultry meat using an electronic nose, *Sensors*. 19 (7) (2019) 1580.
- [28] W. Wójcik, M. Łukasiewicz, K. Puppel, Biogenic amines: formation, action and toxicity – a review, *J. Sci. Food Agric.* 101 (7) (2021) 2634–2640.
- [29] M. Martuscelli, G. Arfelli, A.C. Manetta, G. Suzzi, Biogenic amines content as a measure of the quality of wines of Abruzzo (Italy), *Food Chem.* 140 (3) (2013) 590–597.